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<b>(21) International Application Number:</b> PCT/EP91/01479 <b>(22) International Filing Date:</b> 6 August 1991 (06.08.91) <b>(30) Priority data:</b> A 1668/90 8 August 1990 (08.08.90) AT 683,831 11 April 1991 (11.04.91) US <b>(71) Applicant (for all designated States except US):</b> BIOMAY BIOTECHNIK PRODUKTIONS- UND HANDELSGESELLSCHAFT M.B.H. [AT/AT]; Herrenstraße 2, A-4020 Linz (AT). <b>(72) Inventors; and</b> <b>(73) Inventors/Applicants (for US only):</b> BREITENEDER, Heimo [AT/AT]; Döblinger Hauptstraße 9/1/7, A-1190 Vienna (AT). REIKERSTORFER, Arnold [AT/AT]; Metternichgasse 11/4, A-1030 Vienna (AT). VALENTA, Rudolf [AT/AT]; Beethovenstraße 18, A-2604 Theresienfeld (AT). HOFFMANN-SOMMERGRUBER, Karin [AT/AT]; Titlgasse 4/2, A-1130 Vienna (AT). BREITENBACH, Michael [AT/AT]; Helfertgasse 44, A-1120 Vienna (AT). KRAFT, Dietrich [AT/AT]; Rebenweg 1/18/1, A-1170 Vienna (AT). RUMPOLD, Helmut [AT/AT]; Buchleitengasse 8/3, A-1180 Vienna (AT). SCHEINER, Otto [AT/AT]; Hohe Wandstraße 40, A-2364 Mariaenzersdorf (AT). EBNER, Christof [AT/AT]; St. Elisabethplatz 4/13, A-1040 Vienna (AT). FERREIRA, Fatima [BR/AT]; De Quergasse 6/3, A-1170 Vienna (AT).		<b>(74) Agents:</b> ITZE, Peter et al.; Amerlingstraße 8, A-1061 Vienna (AT).  <b>(81) Designated States:</b> AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), NO, SE (European patent), US.  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>
<b>(54) Title:</b> ALLERGENS OF ALDER POLLEN AND APPLICATIONS THEREOF  <b>(57) Abstract</b>  <p>This invention provides recombinant DNA molecules which code for polypeptides that exhibit the antigenicity of an <i>Aln g</i> I allergen of alder, <i>Alnus sp.</i>, of a <i>Cor a</i> I allergen of hazel or of a <i>Bet v</i> I allergen of birch and other plants of the order Fagales, and for polypeptides comprising at least one epitope thereof, as well as nucleic acids which under stringent conditions hybridize with such DNA sequences or are derivable from such sequences by degeneracy of the genetic code. In addition, methods are described for using the polypeptides coded by these DNA molecules and their use in the diagnosis or therapy of allergic diseases.</p>		

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## ALLERGENS OF ALDER POLLEN AND APPLICATIONS THEREOF

### 1. FIELD OF THE INVENTION

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The invention provides recombinant DNA molecules which code for polypeptides, and the polypeptides per se, that have at least one epitope of an Aln g I pollen allergen, or a Cor a I pollen allergen or a Bet v I pollen allergen of a tree of the order Fagales, particularly alder, Alnus sp., or the entire Aln g I allergen protein, particularly hazel, Corylus sp., or the entire Cor a I allergen protein, or particularly birch, Betula sp., or the entire Bet v I allergen protein, and exhibit the same or similar antigenicity as the Aln g I, the Cor a I or the Bet v I allergen. The invention also provides replicable microbial expression vehicles and microorganisms for use in processes for producing such allergenic polypeptides. Methods are provided for the diagnosis and therapy of allergic diseases using the synthetic polypeptides of the invention.

### 2. BACKGROUND OF THE INVENTION

It has long been known that a type I allergy to pollen proteins is associated with symptoms such as itchy and reddened eyes, running nose, swollen eyelids, coughing and asthmatic conditions. In this respect, the pollens of early-flowering trees of the order Fagales (e.g., birch, hazel, alder and hornbeam) hold an important position. Numerous studies have been carried out to identify and characterize the allergens of these pollens precisely (1 - 4). Progress with regard to the exact characterization of pollen allergens has been hindered by the heterogeneity of the pollen extracts currently in use. Some eight allergens of alder pollen elicit an IgE response in atopics and one of them, Aln g I, a 17 kD protein, reacts with a majority of the sera of allergic patients as the major allergen (5, 6).

At least 10 % of the population suffers from pollen allergies at various times and to varying extent. These allergies are mediated by IgE antibodies which react

with pollen proteins. The possibility exists for a therapy for pollen allergies by hypo-sensitization, i.e., by the regular and slowly increasing administration of the proteins producing the allergy.

Diagnostic methods for allergic diseases, such as radio-allergosorbent test  
5 (RAST), paper radioimmunosorbent test (PRIST), enzyme-linked immunosorbent  
assay (ELISA), radioimmunoassays (RIA), immuno-radiometric assays (IRMA),  
luminescence immunoassays (LIA), histamine release assays, and IgE immunoblots  
depend greatly upon the availability of pure allergens. Protein extracts from pollen  
isolated from natural sources are difficult to standardize because preparations vary  
10 from batch to batch. For example, they may contain unwanted constituents, and/or  
certain proteins may be lost in the extraction procedure and be missing from the final  
preparation (7). Clearly, diagnostic tests which employ well defined allergens that  
can be reproducibly prepared would be superior to tests which employ raw pollen  
extracts with an insufficiently defined mixture of allergens and other components.  
15 Recombinant DNA production of allergenic polypeptides, or allergenic fragments  
thereof, would allow more reproducible preparations of allergens of defined content  
for standardized diagnostic and therapeutic methods.

Allergens may be purified to homogeneity from pollen by known protein/chemi-  
cal methods, for example, by means of affinity chromatography (8). These methods  
20 are relatively costly and require pollen as an expensive source for allergens. It would,  
therefor, be cheaper and more efficient to use recombinant DNA methods to produce  
an allergenic protein, or fragments of that protein.

Hyposensitization has proved to be an effective therapy in allergic diseases.

This therapy consists of parenteral or oral administration of allergens in increasing  
25 doses over a fairly long period of time.

### 3. SUMMARY OF THE INVENTION

The present invention provides recombinant DNA molecules which contain a nucleotide sequence that codes for a polypeptide which exhibits the same or similar 5 antigenic properties as the major allergen, Aln g I, Cor a I or Bet v I of trees of the order Fagales, for example, of alder (Alnus sp.), hazel (Corylus sp.) or birch (Betula sp.) or a polypeptide which comprises at least one epitope of such allergens. The invention provides the complete cDNA sequence of an Aln g I, a Cor a I or a Bet v I allergen and hence the complete deduced amino acid sequences. Additionally, the 10 invention includes (a) nucleotide sequences which hybridize with such a cDNA sequence under high stringency and encode a polypeptide having at least one epitope of an Aln g I, a Cor a I or a Bet v I allergen and (b) nucleotide sequences which can be derived from such allergenic polypeptides by degeneracy of the genetic code. This nucleotide sequence can be expressed as an Aln g I, a Cor a I or a Bet v I allergen, or 15 as a polypeptide which comprises at least one epitope thereof. In a preferred embodiment, this cDNA sequence contains the whole sequence or parts of the sequence set forth in the Sequence Listing as SEQ ID NO. 2 for Aln g I, as SEQ.ID NO.10, 13, 16 and 19 for Cor a I and as SEQ ID NO. 22 for Bet v I.

As concerns their IgE binding, pollens of birch, alder, hazel and hornbeam 20 possess similar major allergens which - so far as is known - exhibit a high degree of homology on the amino acid level. The present invention therefore relates not only to an Aln g I allergen of alder, or Cor a I of hazel or Bet v I of birch, but as well to Aln g I, Cor a I or Bet v I pollen allergens of other species which are coded by DNA allergen under stringent conditions or can be derived from such polypeptide allergens 25 by degeneracy of the genetic code.

Hybridization of a polynucleotide with another polynucleotide under stringent conditions requires at least a 60 % identity between such polynucleotides at the nucleic acid level.

Such stringent conditions entail washing of hybridized nitrocellulose filters as 30 follows:

(a) For DNA/DNA and DNA/RNA hybridizations: A temperature of 55°C, a salt concentration of 150 mM NaCl and 15 mM Na<sub>3</sub> citrate at pH 7,0, and a SDS (Sodium Dodecyl Sulfate) detergent at a concentration of 0,1 % (w/v).

(b) For oligodeoxynucleotide/DNA hybridizations: A temperature of 55°C, a salt concentration of 1M NaCl and 10 mM Na<sub>3</sub>citrate x 2H<sub>2</sub>O at pH 7,0, and a SDS (Sodium Dodecyl Sulfate) detergent at a concentration of 0,5 % (w/v). In this context "oligodeoxynucleotide" refers to an oligomer of a single-stranded DNA of up to 100 nucleotides in length.

In addition, this invention provides expression plasmids that contain a nucleotide sequence as described above and host cells which harbor these expression plasmids.

This invention also provides compositions containing synthetic polypeptides which exhibit the antigenicity of parts or of the whole of an alder Aln g I allergen or of allergens of other plants which, because of a high degree (at least 50 %) of amino acid homology (9), exhibit antigenic cross-reactivity to parts or to all of an alder Aln g I allergen, i.e., antibodies or cellular antigen binding sites which are actually directed to alder Aln g I allergen are likewise able to bind to these molecules. These synthetic polypeptides include fusion and nonfusion polypeptides which contain a polypeptide portion that possesses the antigenicity of a part or of all of an alder polypeptide which contain a polypeptide portion that possesses the antigenicity of a part or of all of an Aln g I, a Cor a I or a Bet v I allergen. The method for preparing such synthetic polypeptides comprises the steps of culturing of prokaryotic or eukaryotic host cells which contain an expression plasmid described above and purification of the synthetic polypeptide(s) from the culture.

25 The term "synthetic" here alternatively includes polypeptides which are prepared by cloning and expression of the nucleotide sequences described here or by chemical synthesis of polypeptides encoded by these nucleotide sequences.

The synthetic polypeptides which are produced according to this invention exhibit antigenicity the same as or similar to the native allergen. As shown below, a 30 cDNA clone coding for an alder Aln g I, a hazel Cor a I or a birch Bet v I can be

used to produce a nonfusion polypeptide which reacts with IgE in the sera of allergic persons. It is therefore possible to use this polypeptide as an antigen in diagnostic tests (such as RAST, PRIST, ELISA, RIA, IRMA, LIA, histamine release assays and IgE immunoblots known in the art and referred to above), as a component of prophylactic or therapeutic agents in hyposensitization therapy, and as a component in any kind of in vivo diagnostic procedure such as bronchial, conjunctival, dermal, nasal and oral provocation and skin tests.

In particular, the synthetic allergens can be used as diagnostic reagents in vitro and in vivo, since their antigenicity corresponds to that of the native Aln g I pollen allergens and they are therefore able to bind IgE of sera of persons suffering from Aln g I pollen allergy. In the same way, the antigenicity corresponds to that of the native Cor a I or Bet v I pollen allergens and they are also able to bind IgE of sera of sensitive or allergic patients.

It is therefore one of the objects of the present invention to provide a method for the preparation of pollen allergens, in particular for Aln g I, Cor a I or Bet v I allergens, so as to have this family of allergens available for diagnostic tests for detection of the corresponding allergy and, alternatively, for hyposensitization therapy.

As main epitopes capable of modifying T-cell response the following amino acid sequences were found:

20 GlyValPheAsnTyrGlu  
PheIleLeuAspGlyAspLysLeu  
AlaIleSerSerValGluAsnIle  
GlyAsnGlyGlyProGlyThrIleLysLysIleSerPhe  
LysTyrValLysAspArgValAspGluValAsp  
25 LeuLeuArgAlaValGluSerTyrLeuLeuAlaHisSer.

All these sequences are present in all said allergens, i.e. Aln g I, Cor a I and Bet v I.

#### 4. BRIEF DESCRIPTION OF THE FIGURES

The following figures and description aid in understanding the field and scope of the invention.

FIG. 1 shows a cDNA (665 nucleotides, SEQ ID NO.1) encompassing the nucleotide sequence encoding an Aln g I allergen of alder. The cDNA sequence consists of a coding region of 483 nucleotides (including the initiation and termination codons), a 3' noncoding region of 162 nucleotides and a poly-A tail of 20 nucleotides. The deduced amino acid sequence of alder Aln g I polypeptide is indicated in FIG. 1 under the respective codons. The complete protein has 160 (SEQ ID NO.3) amino acids (including the methionine of the initiation codon).

FIG. 2 shows the nucleotide sequence of the BP-A primer (SEQ ID NO.4) that was used for synthesis of the first cDNA strand. The recognition sequences of the restriction enzymes BglII (nucleotides 19-24) and HindIII (nucleotides 31-36) are underlined. The sequence of T7 primer (nucleotides 4-17), which was used as primer for the PCR amplification of Aln g I and is the constituent of BPA, is likewise indicated.

FIGS. 3 - 10 show Immunoblot analysis of isoforms of the major hazel pollen allergen Cor a I as recombinant non-fusion proteins, in particular

FIGS. 3 - 6:

An identical set of patients' sera was used to characterize the Cor a I isoforms (lanes 1 - 9).

Lanes B: buffer control without addition of patients' sera.

Lanes N: a pool of non-allergic normal human sera.

IgE antibodies from the allergic patients' sera, which bound to the isoforms, was detected by <sup>125</sup>I labeled rabbit-anti human IgE. Each of the isoforms shows reactivity with IgE from allergic patients' sera. All isoforms were able to bind IgE, although their individual binding pattern may differ from patient to patient.

## FIG. 7:

An identical set of experiments was performed using E.coli JM 105 transformed with the plasmid pKK 223.3 without any cDNA insertion. No bound IgE could be detected.

## FIG. 8:

Likewise the cDNA fragment whose sequence is shown in SEQ ID NO. 1 was ligated into the expression plasmid pKK 223.3. The protein corresponding to the coding region (see SEQ ID NO. 2 and SEQ ID NO.3) was expressed in E.coli JM 105 and tested with the identical set of patients' sera as above. rAln g I was able to bind IgE from these patients' sera in each case (lanes 1 - 9). In lanes B (buffer control, no patients' sera) and N (a pool of sera from non allergic individuals) no binding could be observed.

## FIG. 9:

15 This represents the quality control of the patients' sera used in the above experiments. The very same set of sera was tested on separated and blotted proteins from an aqueous extract of birch pollen. IgE from every single serum bound strongly to the major allergen of birch pollen, Bet v I (lanes 1 - 9). No binding could be observed for the buffer control (lane B) and the pool of sera from non allergic individuals.

## 20 FIG.10:

Furthermore the same sera were tested on rBet v I and showed exactly the same strong reactivity with the recombinant nonfusion protein.

## FIG. 11:

Inhibition experiment showing the capacity of rBet v I to bind IgE from tree 25 pollen allergic patients' sera and thus to prevent the IgE from further binding to the corresponding hazel pollen allergen Cor a I. 1 ml each of a 1 : 10 dilution of birch pollen allergic individuals' sera (1 - 5), of a serum pool of non allergic individuals (6), and buffer without the addition of serum (7) was incubated over night at 4°C with the addition of 5 µg of rBet v I (panel 1), 5 µg of BSA (panel 2), or buffer only 30 (panel 3). These samples were used to probe a Western blot of SDS-PAGE-separated

hazel pollen proteins. In the case where rBet v I had been added no IgE binding to the 17kD Cor a I could be observed. The addition of bovine serum albumin (BSA) or buffer without addition of a protein could not inhibit the binding of patients' IgE to the hazel Cor a I.

5

## 5. EXAMPLES

### 5.1. Poly A+ RNA isolation from pollen and synthesis of the first cDNA strand:

10 Polyadenylated (polyA+) mRNA was isolated from ripe alder pollen (Allergon AB, Engelsholm, Sweden) (1). Using this, the first strand of cDNA was synthesized as follows:

2  $\mu$ l 10x buffer (480 mM Tris (hydroxymethyl) aminomethane (Tris), 60 mM  $MgCl_2$ , 400 mM KCl, pH 4,8)

15 2  $\mu$ l 10 mM dithiothreitol (DTT)

1  $\mu$ l primer BP-A (100 ng/ $\mu$ l, nucleotide sequence of FIG.2) (SEQ ID NO.4)

2  $\mu$ l 25 mM deoxynucleoside triphosphates (dNTPs), i.e. 25 mM each of dATP, dCTP, dGTP, dTTP (Pharmacia, Uppsala, Sweden)

11  $\mu$ l  $H_2O$

20 1  $\mu$ l poly A+RNA (3  $\mu$ g)

1  $\mu$ l AMV reverse transcriptase (United States Biochemical Corporation (USB), Cleveland, Ohio, USA) = 32 Units.

This reaction, with a total volume of 20  $\mu$ l, was incubated for 2 hours at 42°C, then diluted 1 : 1 with 1x TE buffer (10 mM Tris, 1mM ethylenediamine tetraacetic acid (EDTA), pH 8,0) and stored at 4°C.

### 5.2 Polymerase chain reaction (PCR):

PCR was carried out on the hybrid RNA-DNA molecules prepared in Section 5.1. A mixture of the following two oligodeoxynucleotides was used as primer for the 5'-end of the molecules:

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No. 2482 (SEQ ID NO.5)

5'- GTT TTC AAT TAC GAA GCG GAA AC -3'

No.2490 (SEQ ID NO.6)

5'- GTT TTC AAT TAC GAA GCG GAG AC -3'

- 5 The nucleotide sequences of these oligodeoxynucleotides were derived from the N-terminal amino acid sequence of alder Aln g I partially determined by Edman degradation and following the codon usage of birch (B. verrucosa).

T7 primer (SEQ ID NO.7) (Pharmacia), which is likewise a constituent of the BP-A primer, was used as primer for the 3' end of the molecules. The following 10 mixture was used for the reaction:

2,5 µl of the reaction mixture in Section 5.1

5,0 µl 10x PCR buffer (400 mM KCl, 10 mM MgCl<sub>2</sub>, 10 % gelatin, 100 mM Tris, pH 8,3)

2,0 µl T7 primer (SEQ ID NO.7) (Pharmacia) = 20 pmol

- 15 4,0 µl primer mix in equal parts of No. 2482 (SEQ ID NO.5) and 2490 (SEQ ID NO.6) = 100 pmol

2,5 µl 2 mM dNTPs (Pharmacia)

1,5 µl 100 mM MgCl<sub>2</sub>

32,5 µl H<sub>2</sub>O (to 50 µl)

- 20 Addition of 1 unit Taq DNA polymerase (USB). The reaction mixture was incubated for 30 seconds at 93°C, for 30 seconds at 55°C and for 1 minute at 72°C. This cycle was run through 30x in all. Finally, the reaction mixture was kept at 72°C for another 10 minutes.

### 5.3 Cloning of the PCR fragment and sequencing:

- 25 The DNA fragment synthesized in Section 5.2 was isolated from a 1,5 % agarose gel by means of DEAE paper (10). This fragment was then kinased at the 5'-end.

#### a) Kinasing

10 µl DNA (= 500 ng Aln g I DNA)

- 30 2,5 µl 10x T4 polynucleotide kinase buffer (Boehringer, Mannheim, Germany)

7,0  $\mu$ l  $\gamma$ - $^{32}$ P-ATP, 10 mCi/ml (Amersham, Little Chalfont, England)

4,5  $\mu$ l H<sub>2</sub>O

1,0  $\mu$ l polynucleotide kinase (Boehringer)

The reaction mixture was incubated for 20 minutes at 37°C. After that another 5 addition of 1  $\mu$ l polynucleotide kinase was made and the mixture was incubated for 60 minutes at 37°C.

b) Klenow fill-in reaction:

To the above reaction mixture was added:

1  $\mu$ l 2mM dNTPS (Pharmacia)

10 1  $\mu$ l Klenow Fragment (= 2 units)

The kinased and filled-in DNA fragment was purified by way of a Nick™ Column (Pharmacia) and was then precipitated with ethanol and sodium acetate (9).

c) BglII digestion of fragment:

Several restriction enzyme sites were added at the 3'-end to the Aln g I sequence through the use of the BP-A oligodeoxynucleotide (FIG.2; SEQ ID NO.4) in the PCR. The BglII site in this sequence was selected for cleavage with the restriction enzyme, BglII, to ligate the fragment in the corresponding BglII site of pBluescript<sup>®</sup> plasmid (Stratagene, LaJolla, California, USA). Due to the Klenow reaction, blunt ends had already been produced at the 5'-end of the sequence. All the DNA precipitated in Section 5.3b was dissolved in 2  $\mu$ l 10x BglII buffer (Boehringer). 17  $\mu$ l H<sub>2</sub>O and 1  $\mu$ l BglII (11 units) were added. The reaction mixture was incubated for 1,5 hours at 37°C. The fragment so cut was eluted from a 1,5% agarose gel by means of DEAE paper (10).

d) Ligation of the DNA fragment in pBluescript<sup>®</sup> KS+ plasmid:

25 pBluescript<sup>®</sup> KS+ plasmid (Stratagene) was selected as cloning vector and cut with the restriction enzymes EcoRV (supplies flush ends; the 5'-end of the Aln g I fragment is ligated to these) and BamHI (supplies staggered ends compatible with BglII; the 3'-end of the Aln g I fragment is ligated to these). The phosphate groups at the 5'-ends of the plasmid were removed by alkaline phosphatase (12) to prevent non-specific religation of the vector.

30

Ligation of Aln g I fragment in pBluescript<sup>®</sup> KS+ plasmid:

20 ng DNA from Section 5.3c dissolved in 10  $\mu$ l H<sub>2</sub>O

2,0  $\mu$ l 10x ligation buffer (200 mM Tris, 50 mM MgCl<sub>2</sub>, 50 mM DTT, 500  $\mu$ g/ml bovine serum albumin; pH 7,6)

5     1,0  $\mu$ l 10 mM ATP

3,0  $\mu$ l pBluescript<sup>®</sup> KS+ cut with EcoRV and BamHI (= 50 ng)

4,0  $\mu$ l H<sub>2</sub>O

1,0  $\mu$ l T4 DNA ligase Boehringer (= 3 units)

This reaction was incubated for 4 hours at room temperature.

10    e) Transformation of competent E.coli host cells:

Transformation was carried out in E.coli XL1-Blue cells (Stratagene) (13). The selection of positive clones was carried out on ampicillin-containing (100  $\mu$ g/ml) culture plates by means of the blue-white indication system (14).

f) Sequencing of Aln g I DNA:

15    Sequencing of Aln g I DNA was carried out by means of a T7 sequencing kit (Pharmacia), according to the manufacturer's instructions.

#### 5.4 Expression of Aln g I DNA and detection of IgE binding of the resulting proteins:

20    a) The DNA insert from the pBluescript<sup>®</sup> KS+ vector, which contains the coding sequence for Aln g I, was subjected to mutagenesis according to Kunkel et al (15). To complete the Aln g I sequence at the 5'-end and provide it with the ATG codon and an additional EcoRI site, the following oligodeoxynucleotide was synthesized (SEQ ID NO.8): 5'-CTT CGT AAT TGA AAA CAC CCA TGA ATT CCG  
25 ATA CCG TCG A -3' and used for mutagenesis. This enabled the Aln g I sequence to be ligated, in the correct orientation, by means of the EcoRI site at the 5'-end and by means of the HindIII site at the 3'-end of the gene in the expression plasmid pKK 223-3 (Pharmacia). E.coli K12 JM105 cells (thi, rpsL, endA, sbcB15, hsdR4, delta (lacpro AB)/F', thraD36, proAB, lacI<sup>q</sup>Z delta M15) were transformed with this plas-  
30 mid. After protein synthesis was effected, the bacterial cells were harvested and bro-

ken up with liquid nitrogen. The lysate was separated on a SDS polyacrylamide gel. Detection of recombinant Aln g I nonfusion protein was done by means of immunoblot. IgE in the sera of allergic patients was bound by the recombinant Aln g I. Detection of bound IgE was effected by <sup>125</sup>I-labeled antihuman IgE (Pharmacia).

- 5      b) The DNA insert in pBluescript<sup>®</sup> KS+ plasmid, which contains the sequence coding for Aln g I, was ligated by means of EcoRI linkers (Boehringer) in the expression plasmids pEX A, pEX B and pEX C (16), which shift the reading frame of the insert one nucleotide each time. In this way, in one case the correct reading frame for Aln g I was obtained and the production of a recombinant Aln g I fusion protein was  
10 induced. The capability of this recombinant Aln g I fusion protein to bind IgE in sera of patients allergic to alder pollen was shown by means of immunoblot. Detection of bound IgE was effected by <sup>125</sup>I-labeled antihuman IgE (Pharmacia).

An analogous method was applied for the cloning and expressing of Cor a I.

15      5.5 Expression of Cor a I DNA and detection of IgE binding of the resulting protein

The cDNA fragments whose sequences are shown in SEQ ID NO.9, 12, 15 and 18 were ligated into the expression plasmid pKK 223.3 (Pharmacia LKB Biotechnology, Uppsala, Sweden). The proteins corresponding to the coding region (see SEQ ID  
20 NO.10, 13, 16 and 19) of these fragments were expressed in E.coli JM 105 transformed with the respective recombinant plasmids. Cultures were grown until the OD<sub>600</sub> reached 0,4. Isopropyl- $\beta$ -D-galactopyranoside was then added to a final concentration of 0,5 mM and the cultures grown at 37°C over 3,5 hours for expression of recombinant non-fusion proteins. Bacterial cells were harvested by centrifugation, taken up in  
25 50 mM Tris-HCl buffer, pH 7,5, containing 220 mM NaCl and the cells were disrupted by a freezethaw cycle. The supernatant containing the recombinant non-fusion proteins was loaded onto a 15 % SDS-PAGE. The separated proteins were transferred to a nitrocellulose filter. IgE-binding proteins were detected by the use of allergic patients' sera.

30      The results are shown in FIGS. 3 - 6.

### 5.6 Test of reaction of T-cell epitopes

Peripheral blood was collected from birch pollen allergic patients who showed IgE reactivity to Bet v I exclusively, as demonstrated by Western Blot. Peripheral mononuclear cells (PBMC; the white blood cell fraction containing the lymphocytes) were isolated by density gradient centrifugation. Allergen specific T-cells were enriched by culturing PBMC in presence of Bet v I. After a cloning procedure, T-cell clones (TCC) were proved to react with the complete Bet v I molecule by a proliferation assay, showing that in presence of the specific allergen a proliferation occurs, which is at least 10-fold higher than the autoproductive activity of the TCC, as measured by <sup>3</sup>H-Thymidine incorporation. Two Bet v I specific TCC isolated from atopic donors reacted in the same way with the above mentioned peptides as with the whole Bet v I molecule, proving that these peptides represent or contain the relevant T-cell epitopes.

15

TCC	TCC+FC	TCC+FC+ <u>Bet v I</u>	TCC+FC+PEPTIDE	
443	960	30516	31580*	cpm
160	508	21218	23309**	cpm

20 FC: feeder cells

cpm: counts per minute

\* peptide: LLRAVESYLLAHS

\*\*peptide: KYVKDRVDEVD

## 25 6. METHODS OF ADMINISTRATION

The present invention covers the use of the recombinant or synthetic polypeptide allergens to treat a mammal using such polypeptides alone or in combination with any pharmaceutically acceptable carriers or diluents, in accordance with standard pharmaceutical practice.

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The method of treatment involves the administration of such a polypeptide allergen or parts thereof by any route of administration, that is bronchial, conjunctival, dermal, enteral, nasal, oral or vaginal. A range of from 1 picogram to 10 milligrams per application can be used. The diluents and carriers can be chosen by those skilled in the art according to commonly accepted galenic procedures. Like diagnostic methods, it requires pure and well defined allergens. The use of purified recombinant allergens or synthetic peptides would greatly reduce the risk of sensitizing patients to unwanted components.

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## 7. REFERENCES

The references cited in the above specification are:

- 5 1. Breiteneder, H., Pettenburger, K., Bito, A., Valenta, R., Kraft, D., Rumpold, H., Scheiner, O., Breitenbach, M. (1989). The gene coding for the major birch pollen allergen, Bet v I, is highly homologous to a pea resistance response gene. EMBO J., 8: 1935-1938.
- 10 2. Ipsen, H. and Hansen, O.C. (1990). Physicochemical and immunological characteristics of allergens. In: Epitopes of atopic allergens. Schon, A.H., Kraft, D., Kunkel, G. (eds) UCB, Brussels, Belgium, pp 3-8.
- 15 3. Rumpold, H., Rohac, M., Böhle, B., Breitenbach, M., Scheiner, O., Kraft, D. (1990). The relationship of Bet v I epitopes recognized by patients' IgE and monoclonal anti-Bet v I antibodies. In: Epitopes of atopic allergens. Schon, A. H., Kraft, D., Kunkel, G. (eds) UCB, Brussels, Belgium, pp 26-28.
- 20 4. Valenta, R.; Breiteneder, H., Pettenburger, K., Breitenbach, M., Scheiner, O., Kraft, D. (1990). RNA- and DNA-sequence similarities of the

- major allergens of birch, alder, hazel and hornbeam pollens. In: Epitopes of atopic allergens. Schon, A.H., Kraft, D., Kunkel, G. (eds) UCB Brussels, Belgium, pp 73-76.
- 5           5. Florvaag, E. and Elsayed, S. (1984). Comparative studies on tree pollen allergens. VIII. Immunological properties of the alder (*Alnus incana*) pollen extract. Int. Arch. Allergy Appl. Immunol. 75: 300-308.
- 10           6. Florvaag, E., Elsayed, S., Apold, J. (1982). Comparative studies on tree pollen allergens. II. Isolation of alder (*Alnus incana*) pollen allergens: purification and some characteristics of the major allergen pI 4.78. Int. Arch. Allergy Appl. Immunol. 67: 49-56.
- 15           7. Thomas, W.R., Chua, K.Y., Greene, W.K., and Stewart, G.A. (1990). Recombinant mite allergens. In: Epitopes of atopic allergens. Schon, A.H., Kraft, D., and Kunkel, G. (eds). UCB Institute of Allergy, Brussels.
- 20           8. Jarolim, E., Tejkl, M., Rohac, M., Schlerka, G., Breitenbach, M., Scheiner, O., Kraft, D., and Rumpold, H. (1989). Monoclonal antibodies against birch pollen allergens; characterization by immunoblotting and use for single step affinity purification of the major allergen BetvI. Int. Arch. Allergy Appl. Immunol. 90: 54-60.
- 25           9. Needleman, S.B., Wunsch, C.D. (1970). A general method applicable to the search for similarities in the amino acid sequence of two prot ins. J. M l. Biol. 48: 443-453.
- 30           10. Ausubel, F.M. (ed) (1987). Current Protocols in Mol ecular Biology. Green Publishing Associates and Wiley Interscience: John Wil y and Sons, New York. Unit 2.6.
- 35           11. Ibid., Unit 2.1.
12. Ibid., Unit 3.10.

13. Ibid., Unit 1.8.
14. Ibid., Unit 1.
15. Kunkel, T.A., Roberts, J.D. Zakour, R.A.  
(1987). Rapid and efficient site-specific mutagenesis  
5 without phenotypic selection. In: Methods in  
Enzymology Vol. 154. Wu, R. and Grossman, L. (eds)  
Academic Press, Inc. pp 367-382.
16. Nagai, K., Thøgersen, H.C. (1984).  
Generation of  $\delta$ -globin by sequence-specific proteolysis  
10 of a hybrid protein produced in *Escherichia coli*.  
Nature 309: 810-812.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION

- 5 (i) APPLICANT: Dr. Heimo Breiteneder  
Mag. Arnold Reikerstorfer  
Dr. Rudolf Valenta  
Mrs. Dr. Karin Hoffmann -  
Sommergruber  
Dr. Michael Breitenbach  
Dr. Dietrich Kraft  
10 Dr. Helmut Rumpold  
Dr. Otto Scheiner
- (ii) TITLE OF INVENTION: ALLERGENS OF ALDER POLLEN  
AND APPLICATIONS THEREOF
- (iii) NUMBER OF SEQUENCES: 23
- 15 (iv) CORRESPONDENCE ADDRESS:  
(A) ADDRESSEE: Pennie & Edmonds  
(B) STREET: 1155 Avenue of the Americas  
(C) CITY: New York  
(D) STATE: New York  
20 (E) COUNTRY: USA  
(F) ZIP: 10036
- (v) COMPUTER READABLE FORM:  
(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
25 (C) OPERATING SYSTEM: MS-DOS  
(D) SOFTWARE: WordPerfect 5.1
- (vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER: 07/683,831  
(B) FILING DATE: 11-APR-91  
30 (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:  
(A) NAME: Harry C. Jones, III  
(B) REGISTRATION NUMBER: 20,280  
(C) REFERENCE/DOCKET NUMBER: 6530-009
- 35 (ix) TELECOMMUNICATION INFORMATION:  
(A) TELEPHONE: (212) 790-9090  
(B) TELEFAX: (212) 869-9741/8864
- (2) INFORMATION FOR SEQ ID NO: 1:
- 40 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 665 nucleotides  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA of mRNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(vi) ORIGINAL SOURCE:

5

(A) ORGANISM: Alder (Alnus sp.)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
ATGGGTGTTT TCAATTACGA AGCGGAAACC CCTCCGTTA TCCCAGCGGC TCGGCTGTTC 60
AAGGCCTTTA TCCTTGATGG CGATAAGCTC CTTCCAAAGG TTGCACCTGA AGCTGTTAGC 120
AGTGTGAGGA ACATTGAAGG AAATGGAGGG CCTGGAACCA TCAAGAAGAT CACCTTTCCC 180
10 GAAGGCAGCC CTTTAAAGTA CGTAAAGGAG AGGGTTGATG AGGTTGATCG CGTAAACTTC 240
AAATACAGCT TCAGCGTGAT CGAGGGTGGT GCCGTGGGCG ACGCACTGGA GAAGGTCTGT 300
AACGAGATCA AGATAGTGGC AGCCCCTGAT GGAGGATCCA TCTTGAAGAT CAGCAACAAG 360
TTCCACACCA AAGGCGACCA TGAGATAAAT GCAGAGCAGA TTAAGATTGA AAAAGAAAAG 420
GCCGTGGGAC TTCTCAAGGC CGTTGAGAGC TACCTCTTGG CACACTCTGA TGCCTACAAC 480
15 TAAATTCTGC CTAATTTTGA TCAGCTTGCA TGTGTTCTTG TCAAGCCATA AATACTGCTT 540
AACTTCGTCT TGCTAATAAA TGAAGCTGTT GTAGTCGTTT ATGAGTACGT AATAATGACA 600
CCAAACATAT GGAGCCAATT GCTTATGAAT AGAAGTTAAG TTCTTAAAAA AAAAAAAAAA 660
AAAAA 665
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## (3) INFORMATION FOR SEQ ID NO: 2:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 480 nucleotides

(B) TYPE: nucleic acid

5 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA of mRNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

10 (vi) ORIGINAL SOURCE:

(A) ORGANISM: Alder (*Alnus* sp.)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

ATGGGTGTTT TCAATTACGA AGCGGAAACC CCCTCCGTTA TCCCAGCGGC TCGGCTGTTC 60  
AAGGCCTTTA TCCTTGATGG CGATAAGCTC CTTCCAAAGG TTGCACCTGA AGCTGTTAGC 120  
15 AGTGTGAGA ACATTGAAGG AAATGGAGGG CCTGGAACCA TCAAGAAGAT CACCTTTCCC 180  
GAAGGCAGCC CTTTAAAGTA CGTAAAGGAG AGGGTTGATG AGGTTGATCG CGTAAACTTC 240  
AAATACAGCT TCAGCGTGAT CGAGGGTGGT GCCGTGGGCG ACGCACTGGA GAAGGTCTGT 300  
AACGAGATCA AGATAGTGGC AGCCCTGAT GGAGGATCCA TCTTGAAGAT CAGCAACAAG 360  
TTCCACACCA AAGGCGACCA TGAGATAAAT GCAGAGCAGA TTAAGATTGA AAAAGAAAAG 420  
20 GCCGTGGGAC TTCTCAAGGC CGTTGAGAGC TACCTCTTGG CACACTCTGA TGCCTACAAC 480

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## (4) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 160 amino acids

(B) TYPE: amino acid

5 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: polypeptide

(iii) HYPOTHETICAL: No

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Alder (Alnus sp.)

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Met Gly Val Phe Asn Tyr Glu Ala Glu Thr Pro Ser Val Ile Pro Ala  
 1 5 10 15

15 AlaArg Leu Phe Lys Ala Phe Ile Leu Asp Gly Asp Lys Leu Leu Pro  
 20 25 30

Lys Val Ala Pro Glu Ala Val Ser Ser Val Glu Asn Ile Glu Gly Asn  
 35 40 45

20 Gly Gly Pro Gly Thr Ile Lys Lys Ile Thr Phe Pro Glu Gly Ser Pro  
 50 55 60

Phe Lys Tyr Val Lys Glu Arg Val Asp Glu Val Asp Arg Val Asn Phe  
 25 65 70 75 80

Lys Tyr Ser Phe Ser Val Ile Glu Gly Gly Ala Val Gly Asp Ala Leu  
 85 90 95

30 Glu Lys Val Cys Asn Glu Ile Lys Ile Val Ala Ala Pro Asp Gly Gly  
 100 105 110

Ser Ile Leu Lys Ile Ser Asn Lys Phe His Thr Lys Gly Asp His Glu  
 115 120 125

35 Ile Asn Ala Glu Gln Ile Lys Ile Glu Lys Glu Lys Ala Val Gly Leu  
 130 135 140

Leu Lys Ala Val Glu Ser Tyr Leu Leu Ala His Ser Asp Ala Tyr Asn  
 40 145

## (5) INFORMATION FOR SEQ ID N : 4:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 50 nucleotides

(B) TYPE: nucleic acid

5 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other nucleic acid-synthetic

(iii) HYPOTHETICAL: Yes

(ix) FEATURE:

10 (D) OTHER INFORMATION: Primer for reverse  
transcription

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

TTTAATACGA CTCACTATAG ATCTCCCGGG AAGCTTTTTT TTTTTTTTTT 50

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## (6) INFORMATION FOR SEQ ID NO: 5:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 nucleotides

(B) TYPE: nucleic acid

20 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other nucleic acid-synthetic

(iii) HYPOTHETICAL: Yes

(vi) ORIGINAL SOURCE:

25 (C) INDIVIDUAL/ISOLATE: 2482

(ix) FEATURE:

(D) OTHER INFORMATION: Primer for  
polymerase chain reaction (PCR)  
utilized at the 5' end of Aln g I mRNA

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GTTTCAATT ACGAAGCGGA AAC 23

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## (7) INFORMATION FOR SEQ ID NO: 6:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 nucleotides

(B) TYPE: nucleic acid

5 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other nucleic acid-synthetic

(iii) HYPOTHETICAL: Yes

## (vi) ORIGINAL SOURCE:

10 (C) INDIVIDUAL ISOLATE: 2490

## (ix) FEATURE:

(D) OTHER INFORMATION: Primer for  
polymerase chain reaction utilized at  
the 5' end of Aln g I mRNA

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GTTTCAATT ACCAAGCGGA GAC 23

## (8) INFORMATION FOR SEQ ID NO: 7:

## (i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 14 nucleotides

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other nucleic acid-synthetic

25 (iii) HYPOTHETICAL: Yes

## (ix) FEATURE:

(D) OTHER INFORMATION: Primer for  
polymerase chain reaction (PCR)  
utilized at the 3' end of Aln g I mRNA

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

AATACGACTC ACTA 14

## (9) INFORMATION FOR SEQ ID NO: 8:

## (i) SEQUENCE CHARACTERISTICS:

35 (A) LENGTH: 40 nucleotides

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other nucleic acid-synthetic

40 (iii) HYPOTHETICAL: Yes

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

CTTCGTAATT GAAAACACCC ATGAATTCCG ATACCGTCGA 40

## INFORMATION FOR SEQ ID NO: 9

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 619 nucleotides
- 5 (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA of mRNA
- (iii) HYPOTHETICAL: no
- 10 (iv) ANTI-SENSE: no
- (v) FRAGMENT TYPE: not applicable
- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: hazel (*Corylus sp.*)
- (vii) IMMEDIATE SOURCE:
- 15 (A) POLLEN FROM ALLERGON AB, ENGELHOLM, SWEDEN
- (viii) POSITION IN GENOME: not applicable
- (ix) FEATURE: not applicable
- (x) PUBLICATION INFORMATION: not applicable

## 20 (xi) SEQUENCE DESCRIPTION: SEQ. ID NO: 9

```

ATGGGTGTTT TCAATTACGA GGTGAGACT CCCTCCGTTA TCCCTGCGGC      50
AAGGCTGTTT AAGTCCTATG TCCTTGATGG CGATAAGCTC ATCCCAAAGG      100
TTGCACCTCA AGCTATTACC AGCGTTGAAA ACGTTGAAGG AAATGGAGGG      150
25 CCTGGAACCA TCAAGAATAT CACCTTGGC GAAGGCAGCC GTTACAAGTA      200
CGTGAAGGAG AGGGTTGATG AGGTTGACAA CACAACTTC ACATACAGCT      250
ACACCGTGAT CGAGGGTGAT GTCCTGGGTG ACAAGCTGGA GAAGGTCTGC      300
CACGAGCTGA AGATAGTGGC AGCCCTGGT GGAGGATCCA TCTTGAAGAT      350
CAGCAGCAAG TTCCACGCCA AAGGCGACCA TGAGATTAAT GCAGAGGAGA      400
30 TGAAGGGTGC CAAAGAAATG GCAGAGAAC TTTTAAGGGC GGTGAGACC      450
TACCTATTGG CACACTCTGC TGAATACAAC TAAATATCGT CTTGTGTCTT      500
CGCCCAATAA TAACTTGATG GTGGCTTCA TGTTTTTTTT AAAAACTTT      550
GTTTACTTGC TAATAAAGGA GCTTGCGGTT GTGTTTCATCT GCTTGCTGAA      600
AAAAAAAAAA AAAAAAAAAA                                     619

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## INFORMATION FOR SEQ ID NO: 10

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 480 nucleotides
- 5 (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA of mRNA
- (iii) HYPOTHETICAL: no
- 10 (iv) ANTI-SENSE: no
- (v) FRAGMENT TYPE: not applicable
- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: hazel (*Corylus sp.*)
- (vii) IMMEDIATE SOURCE:
- 15 (A) POLLEN FROM ALLERGON AB, ENGELHOLM, SWEDEN
- (viii) POSITION IN GENOME: not applicable
- (ix) FEATURE: not applicable
- (x) PUBLICATION INFORMATION: not applicable
- 20 (xi) SEQUENCE DESCRIPTION: SEQ. ID NO: 10

	ATGGGTGTTT TCAATTACGA GGTGAGACT CCCTCCGTTA TCCCTGCGGC	50
	AAGGCTGTTC AAGTCCTATG TCCTTGATGG CGATAAGCTC ATCCCAAGG	100
	TTGCACCTCA AGCTATTACC AGCGTTGAAA ACGTTGAAGG AAATGGAGGG	150
25	CCTGGAACCA TCAAGAATAT CACCTTTGGC GAAGGCAGCC GTTACAAGTA	200
	CGTGAAGGAG AGGGTTGATG AGGTTGACAA CACAACTTC ACATACAGCT	250
	ACACCGTGAT CGAGGGTGAT GTCCTGGGTG ACAAGCTGGA GAAGGTCTGC	300
	CACGAGCTGA AGATAGTGGC AGCCCCTGGT GGAGGATCCA TCTGAAGAT	350
	CAGCAGCAAG TTCCACGCCA AAGGCGACCA TGAGATTAAT GCAGAGGAGA	400
30	TGAAGGGTGC CAAAGAAATG GCAGAGAAAC TTTTAAGGGC GGTGAGACC	450
	TACCTATTGG CACACTCTGC TGAATACAAC	480

## INFORMATION FOR SEQ ID NO: 11

(i) SEQUENCE CHARACTERISTICS: Cor a I 5 (c)  
 (A) LENGTH: 160 amino acids  
 5 (B) TYPE: amino acid  
 (C) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: polypeptide  
 (iii) HYPOTHETICAL: no  
 (iv) ANTI-SENSE: not applicable  
 10 (v) FRAGMENT TYPE: not applicable  
 (vi) ORIGINAL SOURCE:  
 (A) ORGANISM: hazel (*Corylus sp.*)  
 (vii) IMMEDIATE SOURCE:  
 (A) POLLEN FROM ALLERGON AB, ENGELHOLM, SWEDEN  
 15 (viii) POSITION IN GENOME: not applicable  
 (ix) FEATURE: not applicable  
 (x) PUBLICATION INFORMATION: not applicable  
 (xi) SEQUENCE DESCRIPTION: SEQ. ID NO: 11  
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1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Met	Gly	Val	Phe	Asn	Tyr	Glu	Val	Glu	Thr	Pro	Ser	Val	Ile	Pro
16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
25	Ala	Ala	Arg	Leu	Phe	Lys	Ser	Tyr	Val	Leu	Asp	Gly	Asp	Lys
31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
	Ile	Pro	Lys	Val	Ala	Pro	Gln	Ala	Ile	Thr	Ser	Val	Glu	Asn
30	46	47	48	49	50	51	52	53	54	55	56	57	58	59
	Glu	Gly	Asn	Gly	Gly	Pro	Gly	Thr	Ile	Lys	Asn	Ile	Thr	Phe
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
	Glu	Gly	Ser	Arg	Tyr	Lys	Tyr	Val	Lys	Glu	Arg	Val	Asp	Glu
35	76	77	78	79	80	81	82	83	84	85	86	87	88	89
	Asp	Asn	Thr	Asn	Phe	Thr	Tyr	Ser	Tyr	Thr	Val	Ile	Glu	Gly
91	92	93	94	95	96	97	98	99	100	101	102	103	104	105
40	Val	Leu	Gly	Asp	Lys	Leu	Glu	Lys	Val	Cys	His	Glu	Leu	Lys
106	107	108	109	110	111	112	113	114	115	116	117	118	119	120
	Val	Ala	Ala	Pro	Gly	Gly	Gly	Ser	Ile	Leu	Lys	Ile	Ser	Ser

Val Ala Ala Pro Gly Gly Gly Ser Ile Leu Lys Ile Ser Ser Lys

121 122 123 124 125 126 127 128 129 130 131 132 133 134 135  
Phe His Ala Lys Gly Asp His Glu Ile Asn Ala Glu Glu Met Lys

136 137 138 139 140 141 142 143 144 145 146 147 148 149 150  
5 Gly Ala Lys Glu Met Ala Glu Lys Leu Leu Arg Ala Val Glu Thr

151 152 153 154 155 156 157 158 159 160  
Tyr Leu Leu Ala His Ser Ala Glu Tyr Asn

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## INFORMATION FOR SEQ ID NO: 12

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 742 nucleotides
- 5 (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA of mRNA
- (iii) HYPOTHETICAL: no
- 10 (iv) ANTI-SENSE: no
- (v) FRAGMENT TYPE: not applicable
- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: hazel (*Corylus sp.*)
- (vii) IMMEDIATE SOURCE:
- 15 (A) POLLEN FROM ALLERGON AB, ENGELHOLM, SWEDEN
- (viii) POSITION IN GENOME: not applicable
- (ix) FEATURE: not applicable
- (x) PUBLICATION INFORMATION: not applicable

## 20 (xi) SEQUENCE DESCRIPTION: SEQ. ID NO: 12

```

ATGGGTGTTT TCAATTACGA GGTGAGACT CCCTCCGTTA TCCCAGCGGC      50
AAGGCTGTTT AAGTCCTATG TCCTTGATGG CGATAAGCTC ATCCCAAAGG      100
TTGCACCTCA AGCTATTACC AGCGTTGAAA ACGTTGAAGG AAATGGAGGG      150
25 CCTGGAACCA TCAAGAATAT CACCTTTGGC GAAGGCAGCC GTTACAAGTA      200
CGTGAAGGAG AGGGTTGATG AGGTTGACAA CACAACTTC AAATATAGCT      250
ACACCGTGAT CGAGGGTGAT GTCCTGGGTG ACAAGCTGGA GAAGGTCTGC      300
AGCGAGCTGA AGATAGTGGC AGCCCCTGGT GGAGGATCCA TCTTGAAGAT      350
CAGCAGCAAG TTCCACGCCA AAGGCGACCA TGAGATTAAT GCAGAGGAGA      400
30 TGAAGGGTGC CAAAGAAATG GCCGAGAAAC TTTAAGGGC GGTGAGACC      450
TACCTATTGG CACACTCTGC TGAATACAAC TAAATATCGT CTTGTGTCTT      500
CGCCCAATAA TAACTTGTA C GTGGCTTCA TGTTTTTTTT TTAAACTTT      550
GATTACTTGC TAATAAAGGA GCTTGCGGTT GTGTTTCATCT GCTTGCTGAA      600
ATCGATGTTG TAACTCGGAA GAATGCAAAC TGAATGTTGT ATTACTTTTT      650
35 GCATATATAC AAATAATGGA AAGGATAACA TCATTGAAGT TCAAAAAAAA      700
AAAAA AAAAAA AAAAAA AAAAAA AA      742

```

## INFORMATION FOR SEQ ID NO: 13

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 480 nucleotides  
5     (B) TYPE: nucleic acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear  
    (ii) MOLECULE TYPE: cDNA of mRNA  
    (iii) HYPOTHETICAL: no  
10    (iv) ANTI-SENSE: no  
    (v) FRAGMENT TYPE: not applicable  
    (vi) ORIGINAL SOURCE:  
        (A) ORGANISM: hazel (*Corylus sp.*)  
    (vii) IMMEDIATE SOURCE:  
15     (A) POLLEN FROM ALLERGON AB, ENGELHOLM, SWEDEN  
    (viii) POSITION IN GENOME: not applicable  
    (ix) FEATURE: not applicable  
    (x) PUBLICATION INFORMATION: not applicable  
20 (xi) SEQUENCE DESCRIPTION: SEQ. ID NO: 13

```
ATGGGTGTTT TCAATTACGA GGTGAGACT CCCTCCGTTA TCCCAGCGGC    50
AAGGCTGTTC AAGTCCTATG TCCTTGATGG CGATAAGCTC ATCCCAAAGG    100
TTGCACCTCA AGCTATTACC AGCGTTGAAA ACGTTGAAGG AAATGGAGGG    150
25 CCTGGAACCA TCAAGAATAT CACCTTTGGC GAAGGCAGCC GTTACAAGTA    200
CGTGAAGGAG AGGGTTGATG AGGTTGACAA CACAAACTTC AAATATAGCT    250
ACACCGTGAT CGAGGGTGAT GTCCTGGGTG ACAAGCTGGA GAAGGTCTGC    300
AGCGAGCTGA AGATAGTGGC AGCCCCTGGT GGAGGATCCA TCTTGAAGAT    350
CAGCAGCAAG TTCCACGCCA AAGGCGACCA TGAGATTAAT GCAGAGGAGA    400
30 TGAAGGGTGC CAAAGAAATG GCCGAGAAC TTSTAAGGGC GGTGAGACC    450
TACCTATTGG CACACTCTGC TGAATACAAC                                480
```

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## INFORMATION FOR SEQ ID NO: 14

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 160 amino acids  
 5 (B) TYPE: amino acid  
 (C) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: polypeptide  
 (iii) HYPOTHETICAL: no  
 (iv) ANTI-SENSE: not applicable  
 10 (v) FRAGMENT TYPE: not applicable  
 (vi) ORIGINAL SOURCE:  
 (A) ORGANISM: hazel (*Corylus sp.*)  
 (vii) IMMEDIATE SOURCE:  
 (A) POLLEN FROM ALLERGON AB, ENGELHOLM, SWEDEN  
 15 (viii) POSITION IN GENOME: not applicable  
 (ix) FEATURE: not applicable  
 (x) PUBLICATION INFORMATION: not applicable

(xi) SEQUENCE DESCRIPTION: SEQ. ID NO: 14

20

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15  
 Met Gly Val Phe Asn Tyr Glu Val Glu Thr Pro Ser Val Ile Pro

16 17 18 19 20 21 22 23 24 25 26 27 28 29 30  
 25 Ala Ala Arg Leu Phe Lys Ser Tyr Val Leu Asp Gly Asp Lys Leu

31 32 33 34 35 36 37 38 39 40 41 42 43 44 45  
 Ile Pro Lys Val Ala Pro Gln Ala Ile Thr Ser Val Glu Asn Val

30 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60  
 Glu Gly Asn Gly Gly Pro Gly Thr Ile Lys Asn Ile Thr Phe Gly

61 62 63 64 65 66 67 68 69 70 71 72 73 74 75  
 Glu Gly Ser Arg Tyr Lys Tyr Val Lys Glu Arg Val Asp Glu Val

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76 77 78 79 80 81 82 83 84 85 86 87 88 89 90  
 Asp Asn Thr Asn Phe Lys Tyr Ser Tyr Thr Val Ile Glu Gly Asp

91 92 93 94 95 96 97 98 99 100 101 102 103 104 105  
 40 Val Leu Gly Asp Lys Leu Glu Lys Val Cys Ser Glu Leu Lys Ile

106 107 108 109 110 111 112 113 114 115 116 117 118 119 120  
 Val Ala Ala Pro Gly Gly Gly Ser Il Leu Lys Ile S r Ser Lys

121 122 123 124 125 126 127 128 129 130 131 132 133 134 135  
Phe His Ala Lys Gly Asp His Glu Ile Asn Ala Glu Glu Met Lys

136 137 138 139 140 141 142 143 144 145 146 147 148 149 150  
5 Gly Ala Lys Glu Met Ala Glu Lys Leu Leu Arg Ala Val Glu Thr

151 152 153 154 155 156 157 158 159 160  
Tyr Leu Leu Ala His Ser Ala Glu Tyr Asn

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## INFORMATION FOR SEQ ID NO: 15

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 655 nucleotides
- 5 (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA of mRNA
- (iii) HYPOTHETICAL: no
- 10 (iv) ANTI-SENSE: no
- (v) FRAGMENT TYPE: not applicable
- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: hazel (*Corylus sp.*)
- (vii) IMMEDIATE SOURCE:
- 15 (A) POLLEN FROM ALLERGON AB, ENGELHOLM, SWEDEN
- (viii) POSITION IN GENOME: not applicable
- (ix) FEATURE: not applicable
- (x) PUBLICATION INFORMATION: not applicable

## 20 (xi) SEQUENCE DESCRIPTION: SEQ. ID NO: 15

```

ATGGGTGTTT TCAATTACGA GGCTGAGACC ACCTCCGTTA TCCCTGCGGC      50
AAGGCTGTTT AAGTCCTATG TCCTTGATGG CGATAAGCTC ATCCCAAAGG      100
TTGCACCTCA AGCTATTACC AGCGTTGAAA ACGTTGAAGG AAATGGAGGG      150
25 CCTGGAACCA TCAAGAATAT CACCTTTGGC GAAGGCAGCC GTTACAAGTA      200
CGTGAAGGAG AGGGTTGATG AGGTTGACAA CACAACTTC ACATACAGCT      250
ACACCGTGAT CGAGGGTGAT GTCCTGGGTG ACAAGCTGGA GAAGGTCTGC      300
CACGAGCTGA AGATAGTGGC AGCCCCTGGT GGAGGATCCA TCTTGAAGAT      350
CAGCAGCAAG TTCCACGCCA AAGGTGACCA TGAGATTAAAT GCAGAGGAGA      400
30 TGAAGGGTGC CAAAGAAATG GCCGAGAAAC TTTTAAGGGC GGTGAGACC      450
TACCTATTGG CACACTCTGC TGAATACAAC TAAACCTCGT CTTGTGTCTT      500
CGCCCAATAA TAGCTTGTAAC GTGGCTTCA TGTTTTTTTT TAAACTTTG      550
TTTTCTTGCT AATAAAGGAG CTTGCGGTTG TGTTTCATCTG CTTGCTGAAG      600
ATCGATGTTG TAACTCGGAA GAATGCAAAT TTAATGTTGT ATTAAAAAAA      650
35 AAAAAA                                     655

```

## INFORMATION FOR SEQ ID NO: 16

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 480 nucleotides
  - (B) TYPE: nucleic acid
  - 5 (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: cDNA of mRNA
  - (iii) HYPOTHETICAL: no
  - (iv) ANTI-SENSE: no
  - 10 (v) FRAGMENT TYPE: not applicable
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: hazel (*Corylus sp.*)
  - (vii) IMMEDIATE SOURCE:
    - (A) POLLEN FROM ALLERGON AB, ENGELHOLM, SWEDEN
  - 15 (viii) POSITION IN GENOME: not applicable
  - (ix) FEATURE: not applicable
  - (x) PUBLICATION INFORMATION: not applicable

## (xi) SEQUENCE DESCRIPTION: SEQ. ID NO: 16

20  
ATGGGTGTTT TCAATTACGA GGCTGAGACC ACCTCCGTTA TCCCTGCGGC 50  
AAGGCTGTTC AAGTCCTATG TCCTTGATGG CGATAAGCTC ATCCCAAGG 100  
TTGCACCTCA AGCTATTACC AGCGTTGAAA ACGTTGAAGG AAATGGAGGG 150  
CCTGGAACCA TCAAGAATAT CACCTTTGGC GAAGGCAGCC GTTACAAGTA 200  
25 CGTGAAGGAG AGGGTTGATG AGGTTGACAA CACAACTTC ACATACAGCT 250  
ACACCGTGAT CGAGGGTGAT GTCCTGGGTG ACAAGCTGGA GAAGGTCTGC 300  
CACGAGCTGA AGATAGTGGC AGCCCCTGGT GGAGGATCCA TCTTGAAGAT 350  
CAGCAGCAAG TTCCACGCCA AAGGTGACCA TGAGATTAAT GCAGAGGAGA 400  
TGAAGGGTGC CAAAGAAATG GCGAGAAAC TTTTAAGGGC GGTGAGACC 450  
30 TACCTATTGG CACACTCTGC TGAATACAAC 480

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## INFORMATION FOR SEQ ID NO: 17

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 160 amino acids
  - (B) TYPE: amino acid
  - (C) TOPOLOGY: linear
- (ii) MOLECULE TYPE: polypeptide
- (iii) HYPOTHETICAL: no
- (iv) ANTI-SENSE: not applicable
- (v) FRAGMENT TYPE: not applicable
- 10 (vi) ORIGINAL SOURCE:
- (A) ORGANISM: hazel (*Corylus sp.*)
- (vii) IMMEDIATE SOURCE:
- (A) POLLEN FROM ALLERGON AB, ENGELHOLM, SWEDEN
- (viii) POSITION IN GENOME: not applicable
- 15 (ix) FEATURE: not applicable
- (x) PUBLICATION INFORMATION: not applicable
- (xi) SEQUENCE DESCRIPTION: SEQ. ID NO: 17

```

20  1   2   3   4   5   6   7   8   9  10  11  12  13  14  15
    Met Gly Val Phe Asn Tyr Glu Ala Glu Thr Thr Ser Val Ile Pro

    16  17  18  19  20  21  22  23  24  25  26  27  28  29  30
    Ala Ala Arg Leu Phe Lys Ser Tyr Val Leu Asp Gly Asp Lys Leu

25  31  32  33  34  35  36  37  38  39  40  41  42  43  44  45
    Ile Pro Lys Val Ala Pro Gln Ala Ile Thr Ser Val Glu Asn Val

    46  47  48  49  50  51  52  53  54  55  56  57  58  59  60
30  Glu Gly Asn Gly Gly Pro Gly Thr Ile Lys Asn Ile Thr Phe Gly

    61  62  63  64  65  66  67  68  69  70  71  72  73  74  75
    Glu Gly Ser Arg Tyr Lys Tyr Val Lys Glu Arg Val Asp Glu Val

    76  77  78  79  80  81  82  83  84  85  86  87  88  89  90
35  Asp Asn Thr Asn Phe Thr Tyr Ser Tyr Thr Val Ile Glu Gly Asp

    91  92  93  94  95  96  97  98  99 100 101 102 103 104 105
    Val Leu Gly Asp Lys Leu Glu Lys Val Cys His Glu Leu Lys Ile

40 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
    Val Ala Ala Pr  Gly Gly Gly Ser Ile Leu Lys Ile S r Ser Lys

    121 122 123 124 125 126 127 128 129 130 131 132 133 134 135

```

Phe His Ala Lys Gly Asp His Glu Ile Asn Ala Glu Glu Met Lys

136 137 138 139 140 141 142 143 144 145 146 147 148 149 150  
Gly Ala Lys Glu Met Ala Glu Lys Leu Leu Arg Ala Val Glu Thr

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151 152 153 154 155 156 157 158 159 160  
Tyr Leu Leu Ala His Ser Ala Glu Tyr Asn

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## INFORMATION FOR SEQ ID NO: 18

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 860 nucleotides
- 5 (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA of mRNA
- (iii) HYPOTHETICAL: no
- 10 (iv) ANTI-SENSE: no
- (v) FRAGMENT TYPE: not applicable
- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: hazel (*Corylus sp.*)
- (vii) IMMEDIATE SOURCE:
- 15 (A) POLLEN FROM ALLERGON AB, ENGELHOLM, SWEDEN
- (viii) POSITION IN GENOME: not applicable
- (ix) FEATURE: not applicable
- (x) PUBLICATION INFORMATION: not applicable

## 20 (xi) SEQUENCE DESCRIPTION: SEQ. ID NO: 18

```

ATGGGTGTTT TCAATTACGA GGTTGAGACC CCCTCCGTTA TCTCAGCGGC      50
AAGGCTGTTT AAGTCCTATG TCCTTGATGG CGATAAGCTC ATCCCAAAGG      100
TTGCACCTCA AGCTATTACC AGCGTTGAAA ACGTTGGAGG AAATGGAGGG      150
25 CCTGGAACCA TCAAGAATAT CACCTTTGGC GAAGGCAGCC GTTACAAGTA      200
CGTGAAGGAG AGGGTTGATG AGGTTGACAA CACAACTTC AAATATAGCT      250
ACACCGTGAT CGAGGGTGAT GTCCTGGGTG ACAAGCTGGA GAAAGTCTGC      300
AGCGAGCTGA AGTAGTGGC AGCCCCTGGT GGGGGATCCA CCTTGAAGAT      350
CAGCAGCAAG TTCCACGCCA AAGGTGACCA TGAGATTAAT GCAGAGGAGA      400
30 TGAAGGGTGC CAAAGAAATG GCCGAGAAAC TTTAAGGGC GGTGAGACC      450
TACCTATTGG CACACTCTGC TGAATACAAC TAAATATCGT CTTGTGTCTT      500
CGCCAATAAT AACTTGTAGG TGGCTTTCAT GTTTTTTTTT AAAAACTTT      550
GTTTACTTGC TAATAAAGGA GCTGCGGTT GTGTTCACTT GCTTGCTGAA      600
ATCGATGTTG TAACTCGGAA GAATGCAAAC TGAATGTTGT ATTACTTTTT      650
35 GCATATATAC AAATAATGGA AAGGATAACA TCATTGAAGT TCAAAAAAAA      700
GAAAAAAGAA AGCTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTGTCA      750
ATTTAACCC GATACTGATA CTCAAAAATG CAAGAGAGTT TCCGCATAAG      800
CACAAATTTG TTATGTTGAC TTAATACATT ATAAGCAAAA AAAAAAAGAA      850
AAAAAAGAAA

```

## INFORMATI N FOR SEQ ID NO: 19

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 480 nucleotides
- 5 (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA of mRNA
- (iii) HYPOTHETICAL: no
- 10 (iv) ANTI-SENSE: no
- (v) FRAGMENT TYPE: not applicable
- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: hazel (*Corylus sp.*)
- (vii) IMMEDIATE SOURCE:
- 15 (A) POLLEN FROM ALLERGON AB, ENGELHOLM, SWEDEN
- (viii) POSITION IN GENOME: not applicable
- (ix) FEATURE: not applicable
- (x) PUBLICATION INFORMATION: not applicable
- 20 (xi) SEQUENCE DESCRIPTION: SEQ. ID NO: 19

```

ATGGGTGTTT TCAATTACGA GGTGAGACC CCCTCCGTTA TCTCAGCGGC      50
AAGGCTGTTT AAGTCCTATG TCCTTGATGG CGATAAGCTC ATCCCAAAGG     100
TTGCACCTCA AGCTATTACC AGCGTTGAAA ACGTTGGAGG AAATGGAGGG     150
25 CCTGGAACCA TCAAGAATAT CACCTTTGGC GAAGGCAGCC GTTACAAGTA     200
CGTGAAGGAG AGGGTTGATG AGGTTGACAA CACAACTTC AAATATAGCT     250
ACACCGTGAT CGAGGGTGAT GTCCTGGGTG ACAAGCTGGA GAAAGTCTGC     300
AGCGAGCTGA AGATAGTGGC AGCCCCTGGT GGGGGATCCA CCTTGAAGAT     350
CAGCAGCAAG TTCCACGCCA AAGGTGACCA TGAGATTAAT GCAGAGGAGA     400
30 TGAAGGGTGC CAAAGAAATG GCCGAGAAAC TTTTAAGGGC GGTGAGACC     450
TACCTATTGG CACACTCTGC TGAATACAAC                                480

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## INFORMATION FOR SEQ ID NO: 20

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 160 amino acids
  - 5 (B) TYPE: amino acid
  - (C) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: polypeptide
  - (iii) HYPOTHETICAL: no
  - (iv) ANTI-SENSE: not applicable
  - 10 (v) FRAGMENT TYPE: not applicable
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: hazel (*Corylus sp.*)
  - (vii) IMMEDIATE SOURCE:
    - (A) POLLEN FROM ALLERGON AB, ENGELHOLM, SWEDEN
  - 15 (viii) POSITION IN GENOME: not applicable
  - (ix) FEATURE: not applicable
  - (x) PUBLICATION INFORMATION: not applicable
  - (xi) SEQUENCE DESCRIPTION: SEQ. ID NO: 20
- 20
- |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 15  |
| Met | Gly | Val | Phe | Asn | Tyr | Glu | Val | Glu | Thr | Pro | Ser | Val | Ile | Ser |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 16  | 17  | 18  | 19  | 20  | 21  | 22  | 23  | 24  | 25  | 26  | 27  | 28  | 29  | 30  |
| 25  | Ala | Ala | Arg | Leu | Phe | Lys | Ser | Tyr | Val | Leu | Asp | Gly | Asp | Lys |
| 31  | 32  | 33  | 34  | 35  | 36  | 37  | 38  | 39  | 40  | 41  | 42  | 43  | 44  | 45  |
| Ile | Pro | Lys | Val | Ala | Pro | Gln | Ala | Ile | Thr | Ser | Val | Glu | Asn | Val |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 30  | 46  | 47  | 48  | 49  | 50  | 51  | 52  | 53  | 54  | 55  | 56  | 57  | 58  | 59  |
| Gly | Gly | Asn | Gly | Gly | Pro | Gly | Thr | Ile | Lys | Asn | Ile | Thr | Phe | Gly |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 61  | 62  | 63  | 64  | 65  | 66  | 67  | 68  | 69  | 70  | 71  | 72  | 73  | 74  | 75  |
| Glu | Gly | Ser | Arg | Tyr | Lys | Tyr | Val | Lys | Glu | Arg | Val | Asp | Glu | Val |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 35  | 76  | 77  | 78  | 79  | 80  | 81  | 82  | 83  | 84  | 85  | 86  | 87  | 88  | 89  |
| Asp | Asn | Thr | Asn | Phe | Lys | Tyr | Ser | Tyr | Thr | Val | Ile | Glu | Gly | Asp |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 91  | 92  | 93  | 94  | 95  | 96  | 97  | 98  | 99  | 100 | 101 | 102 | 103 | 104 | 105 |
| 40  | Val | Leu | Gly | Asp | Lys | Leu | Glu | Lys | Val | Cys | Ser | Glu | Leu | Lys |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 106 | 107 | 108 | 109 | 110 | 111 | 112 | 113 | 114 | 115 | 116 | 117 | 118 | 119 | 120 |
| Val | Ala | Ala | Pro | Gly | Gly | Gly | Ser | Thr | Leu | Lys | Ile | Ser | Ser | Lys |

121 122 123 124 125 126 127 128 129 130 131 132 133 134 135  
Phe His Ala Lys Gly Asp His Glu Ile Asn Ala Glu Glu Met Lys

136 137 138 139 140 141 142 143 144 145 146 147 148 149 150  
5 Gly Ala Lys Glu Met Ala Glu Lys Leu Leu Arg Ala Val Glu Thr

151 152 153 154 155 156 157 158 159 160  
Tyr Leu Leu Ala His Ser Ala Glu Tyr Asn

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## INFORMATION FOR SEQ ID NO: 21

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 672 nucleotides  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA of mRNA  
(iii) HYPOTHETICAL: no  
(iv) ANTI-SENSE: no  
(v) FRAGMENT TYPE: not applicable  
(vi) ORIGINAL SOURCE:  
(A) ORGANISM: birch (*Betula sp.*)  
(vii) IMMEDIATE SOURCE:  
(A) POLLEN FROM ALLERGON AB, ENGELHOLM, SWEDEN  
(viii) POSITION IN GENOME: not applicable  
(ix) FEATURE: not applicable  
(x) PUBLICATION INFORMATION: not applicable

## 20 (xi) SEQUENCE DESCRIPTION: SEQ. ID NO: 21

```
ATGGGTGTTT TCAATTACGA AACTGAGACC ACCTCTGTTA TCCCAGCAGC    50
TCGACTGTC AAGGCCTTTA TCCTTGATGG CGATAATCTC TTTCCAAAGG    100
TTGCACCCCA AGCCATTAGC AGTGTGAAA ACATTGAAGG AAATGGAGGG    150
25 CCTGGAACCA TTAAGAAGAT CAGCTTTCCC GAAGGCTTCC CTTTCAAGTA    200
CGTGAAGGAC AGAGTTGATG AGGTGGACCA CACAACTTC AAATACAATT    250
ACAGCGTGAT CGAGGGCGGT CCCATAGGCG ACACATTGGA GAAGATCTCC    300
AACGAGATAA AGATAGTGGC AACCCTGAT GGAGGATCCA TCTTGAAGAT    350
CAGCAACAAG TACCACACCA AAGGTGACCA TGAGGTGAAG GCAGAGCAGG    400
30 TTAAGGCAAG TAAAGAAATG GGCGAGACAC TTTTGAGGGC CGTTGAGAGC    450
TACCTCTTGG CACACTCCGA TGCCTACAAC TAATTAATTA ACTTGTGTGC    500
TCTCGAACAT GTCCCTGATC AATAATGGGT TGCAGTGTTT ATGGTGTGTT    550
TTGGGTCTAA TAAAGGAGCT TGCAGTTGTG ATCATCTGCT TGCTAGCTGA    600
AGATGTTGTA ATTTATTGGG AGAATGATAA TAAATGTTCT ATTAAAAAAA    650
35 AAAAAAAAAA AAAAAAAAAA AA                                672
```

## INFORMATION FOR SEQ ID NO: 22

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 480 nucleotides
  - (B) TYPE: nucleic acid
  - 5 (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: cDNA of mRNA
  - (iii) HYPOTHETICAL: no
  - (iv) ANTI-SENSE: no
  - 10 (v) FRAGMENT TYPE: not applicable
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: birch (*Betula sp.*)
  - (vii) IMMEDIATE SOURCE:
    - (A) POLLEN FROM ALLERGO AB, ENGELHOLM, SWEDEN
  - 15 (viii) POSITION IN GENOME: not applicable
  - (ix) FEATURE: not applicable
  - (x) PUBLICATION INFORMATION: not applicable
  - (xi) SEQUENCE DESCRIPTION: SEQ. ID NO: 22

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```

ATGGGTGTTT TCAATTACGA AACTGAGACC ACCTCTGTTA TCCCAGCAGC    50
TCGACTGTTC AAGGCCTTTA TCCTTGATGG CGATAATCTC TTTCCAAAGG    100
TTGCACCCCA AGCCATTAGC AGTGTGAAA ACATTGAAGG AAATGGAGGG    150
25 CCTGGAACCA TTAAGAAGAT CAGCTTTCCC GAAGGCTTCC CTTTCAAGTA    200
CGTGAAGGAC AGAGTTGATG AGGTGGACCA CACAACTTC AAATACAATT    250
ACAGCGTGAT CGAGGGCGGT CCCATAGGCG ACACATTGGA GAAGATCTCC    300
AACGAGATAA AGATAGTGGC AACCCTGAT GGAGGATCCA TCTTGAAGAT    350
CAGCAACAAG TACCACACCA AAGGTGACCA TGAGGTGAAG GCAGAGCAGG    400
30 TTAAGGCAAG TAAAGAAATG GCGGAGACAC TTTGAGGGC CGTTGAGAGC    450
TACCTCTTGG CACACTCCGA TGCCTACAAC    480

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## INFORMATION FOR SEQ ID NO: 23

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 160 amino acids  
 (B) TYPE: amino acid  
 5 (C) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: polypeptide  
 (iii) HYPOTHETICAL: no  
 (iv) ANTI-SENSE: not applicable  
 (v) FRAGMENT TYPE: not applicable  
 10 (vi) ORIGINAL SOURCE:  
 (A) ORGANISM: birch (*Betula sp.*)  
 (vii) IMMEDIATE SOURCE:  
 (A) POLLEN FROM ALLERGON AB, ENGELHOLM, SWEDEN  
 (viii) POSITION IN GENOME: not applicable  
 15 (ix) FEATURE: not applicable  
 (x) PUBLICATION INFORMATION: not applicable  
 (xi) SEQUENCE DESCRIPTION: SEQ. ID NO: 23

```

20  1  2  3  4  5  6  7  8  9 10 11 12 13 14 15
    Met Gly Val Phe Asn Tyr Glu Thr Glu Thr Thr Ser Val Ile Pro

    16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
    Ala Ala Arg Leu Phe Lys Ala Phe Ile Leu Asp Gly Asp Asn Leu

25  31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
    Phe Pro Lys Val Ala Pro Gln Ala Ile Ser Ser Val Glu Asn Ile

    46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
30  Glu Gly Asn Gly Gly Pro Gly Thr Ile Lys Lys Ile Ser Phe Pro

    61 62 63 64 65 66 67 68 69 70 71 72 73 74 75
    Glu Gly Phe Pro Phe Lys Tyr Val Lys Asp Arg Val Asp Glu Val

35  76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
    Asp His Thr Asn Phe Lys Tyr Asn Tyr Ser Val Ile Glu Gly Gly

    91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
    Pro Ile Gly Asp Thr Leu Glu Lys Ile Ser Asn Glu Ile Lys Ile

40  106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
    Val Ala Thr Pro Asp Gly Gly Ser Ile Leu Lys Ile S r Asn Lys

    121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
  
```

Tyr His Thr Lys Gly Asp His Glu Val Lys Ala Glu Gln Val Lys

136 137 138 139 140 141 142 143 144 145 146 147 148 149 150

Ala Ser Lys Glu Met Gly Glu Thr Leu Leu Arg Ala Val Glu Ser

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151 152 153 154 155 156 157 158 159 160

Tyr Leu Leu Ala His Ser Asp Ala Tyr Asn

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## CLAIMS

1. A recombinant DNA molecule, comprising a DNA coding for a polypeptide having at least one epitope of an allergen of trees of the order Fagales, the allergen is 5 selected from the group Aln g I, Cor a I or Bet v I.

2. A recombinant DNA molecule according to claim 1, wherein the allergen Aln g I, Cor a I or Bet v I is selected from the group consisting of birch, alder, hazel and hornbeam.

3. A recombinant DNA molecule according to claim 1, wherein the epitopes of 10 the allergens having an amino acid sequence selected from the following group

GlyValPheAsnTyrGlu

PhelleLeuAspGlyAspLysLeu

AlaIleSerSerValGluAsnIle

GlyAsnGlyGlyProGlyThrIleLysLysIleSerPhe

15 LysTyrValLysAspArgValAspGluValAsp

LeuLeuArgAlaValGluSerTyrLeuLeuAlaHisSer

4. A recombinant DNA molecule according to claim 1 having at least 60% identity to the nucleotid sequence as shown in SEQ ID No. 2.

5. A recombinant DNA molecule according to claim 1, wherein the allergen is 20 an Aln g I allergen of alder.

6. A recombinant DNA molecule according to claim 1, 2, 3, 4 or 5 which codes for a polypeptide having the entire amino acid sequence of an Aln g I allergen.

7. A recombinant DNA molecule according to claim 5, which codes for a polypeptide having all or part of the amino acid sequence as defined in the Sequence 25 Listing by SEQ ID NO:3.

8. A recombinant DNA molecule according to claim 1, wherein the allergen is a Cor a I allergen of hazel.

9. A recombinant DNA molecule according to claim 1 or 8, which codes for a polypeptide having the entire amino acid sequence of a Cor a I allergen.

10. A recombinant DNA molecule according to claim 1 having at least 60% identity to the nucleotid sequence as shown in SEQ ID NOs. 10, 13, 16, and 19.

11. A recombinant DNA molecule according to claim 8 which codes for a polypeptide having all or part of the amino acid sequence as defined in the Sequence Listing by SEQ ID NOs. 11, 14, 17, and 20.

12. A recombinant DNA molecule according to claim 1 wherein the allergen is a Bet v I allergen of birch.

13. A recombinant DNA molecule according to claim 1 having at least 60% identity to the nucleotid sequence as shown in SEQ ID No. 22.

10 14. A recombinant DNA molecule according to claim 12, which codes for a polypeptide having all or part of the amino acid sequence as defined in the Sequence Listing by SEQ ID NO. 23.

15. A recombinant DNA molecule according to claim 3 which codes for one of the epitopes of the allergens as listed in claim 3.

15 16. A polypeptide having at least one epitope of an Aln g I, a Cor a I or a Bet v I allergen showing the same or a similar capacity to bind IgE from tree pollen allergic individual's sera.

17. A replicable prokaryotic or eukaryotic expression vehicle capable, in a transformant prokaryotic or eukaryotic host organism, of being replicated and of directing expression of a DNA of claim 1 to 15 to produce said polypeptides.

18. A prokaryotic or eukaryotic host organism transformed with an expression vehicle capable, in said host organism, of being replicated and of directing expression of a DNA of claim 1 to 15 to produce said polypeptides.

19. A host organism according to claim 18, wherein the organism is Escherichia coli.

20. A method for producing a polypeptide having at least one epitope of an Aln g I, a Cor a I or a Bet v I allergen, comprising culturing a prokaryotic or eukaryotic host organism containing an expression vehicle capable, in said host organism, of being replicated and of directing expression of a DNA of claim 1 to 15 to produce said polypeptides.

21. A composition comprising a polypeptide having at least one epitope of an Aln g I, a Cor a I or a Bet v I allergen and produced by a method according to claim 20.

5 22. A composition comprising a polypeptide having at least one epitope of an Aln g I allergen and produced by a chemical synthesis according to amino acid sequence as defined in the Sequence Listing by SEQ ID NO. 3.

23. A composition comprising a polypeptide having at least one epitope of a Cor a I allergen and produced by a chemical synthesis according to amino acid sequence as defined in the Sequence Listing SEQ ID NOs. 11, 14, 17 and 20.

24. A composition comprising a polypeptide having at least one epitope of a Bet v I allergen and produced by chemical synthesis according to the amino acid sequence as defined in the Sequence Listing by SEQ ID No. 23.

25. An isolated allergenic peptide of pollen of trees of the order Fagales, having at least one of the epitopes with amino acid sequence listed in claim 3.

26. A peptide according to claim 6, 7, 8, 9, 11, 12, 14, or 25, capable of modifying in a sensitive individual to whom it is administered, an allergic response to a pollen of a tree of the order Fagales.

27. A peptide according to claim 26 capable of modifying T-cell response to a pollen of trees of the order Fagales.

28. An isolated peptide of the claim 6, 7, 8, 9, 11, 12, 14 or 25 capable of interfering with an allergic response.

29. A method for detecting IgE antibodies comprising contacting serum of a mammal with a composition according to claim 21, and detecting any immunological reaction between IgE antibodies in the serum and said polypeptide having at least one epitope of an Aln g I, a Cor a I or a Bet v I allergen.

30. A method for detecting allergic reactions to an Aln g I, a Cor a I or a Bet v I allergen, comprising challenging a mammal with a composition according to claim 21 so as to elicit bronchial, conjunctival, dermal, nasal or oral provocation of said

mammal, and detecting any immunological reaction between said tissues and said polypeptides.

31. A method for detecting in vitro a cellular reaction to an Aln g I, a Cor a I or a Bet v I allergen , comprising contacting mammalian cells with a composition 5 according to claim 21, and detecting any reaction between said cells and said polypeptide.

32. A method for treating a mammal afflicted with a pollen allergy, comprising administering an effective amount of a composition according to claim 21 to hyposen-  
sitize said mammal to an Aln g I, a Cor a I or a Bet v I allergen.

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Aln g I

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1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
ATG GGT TTC AAT TAC GAA GCG GAA ACC CCC TCC GTT ATC CCA GCG GCT CGG CTG TTC
Met Gly Val Phe Asn Tyr Glu Ala Glu Thr Pro Ser Val Ile Pro Ala Ala Arg Leu Phe

21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40
AAG GCC TTT ATC CTT GAT GGC GAT AAG CTC CTT CCA AAG GTT GCA CCT GAA GCT GTT AGC
Lys Ala Phe Ile Leu Asp Gly Asp Lys Leu Leu Pro Lys Val Ala Pro Glu Ala Val Ser

41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
AGT GTT GAG AAC ATT GAA GGA AAT GGA GGG CCT GGA ACC ATC AAG AAG ATC ACC TTT CCC
Ser Val Glu Asn Ile Glu Gly Asn Gly Gly Pro Gly Thr Ile Lys Lys Ile Thr Phe Pro

61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80
GAA GGC AGC CCT TTT AAG TAC GTA AAG GAG AGG GTT GAT GAG GTT GAT CGC GTA AAC TTC
Glu Gly Ser Pro Phe Lys Tyr Val Lys Glu Arg Val Asp Glu Val Asp Arg Val Asn Phe

81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100
AAA TAC AGC TTC AGC GTG ATC GAG GGT GGT GCC GTG GGC GAC GCA CTG GAG AAG GTC TGT
Lys Tyr Ser Phe Ser Val Ile Glu Gly Gly Ala Val Gly Asp Ala Leu Glu Lys Val Cys

101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
AAC GAG ATC AAG ATA GTG GCA GCC CCT GAT GGA GGA TCC ATC ATC TTG AAG ATC AGC AAC AAG
Asn Glu Ile Lys Ile Val Ala Ala Pro Asp Gly Gly Ser Ile Leu Lys Ile Ser Asn Lys

121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140
TTC CAC ACC AAA GGC GAC CAT GAG ATA AAT GCA GAG CAG CAG ATT AAG ATT GAA AAA GAA AAG
Phe His Thr Lys Lys Gly Asp His Glu Ile Asn Ala Glu Glu Gln Ile Lys Ile Glu Lys Glu Lys

141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160
GCC GTG GGA CTT CTC AAG GCC GTT GAG AGC TAC CTC TTT GCA CAC CAC TCT GAT GCC TAC AAC
Ala Val Gly Leu Leu Lys Ala Val Glu Ser Tyr Leu Leu Ala His Ser Asp Ala Tyr Asn

161
TAA ATTCTGCCTAATTTTGATCAGCTTGTCATGTGTCTTGTCAAGCCATAAATACTGCTTAACCTTCGTTGCTAATA
End
AATGAAGCTGTTGTAGTCGTTTATGAGTACGTAATAATGACACCAACAATATGGAGCCCAATTGCTTATGAAATAGAGTT
AAGTCTTAAAAAATAAAAAAAAAAAAAA

```

Figur 1

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FIGURE 2

1                    11                    21                    31                    41  
5'-TTTAATACGA CTCACTATAG ATCCCCGG AAGCTTTTT TTTTTTTTTT-3'  
                  T7 Primer                    BglII                    HindIII

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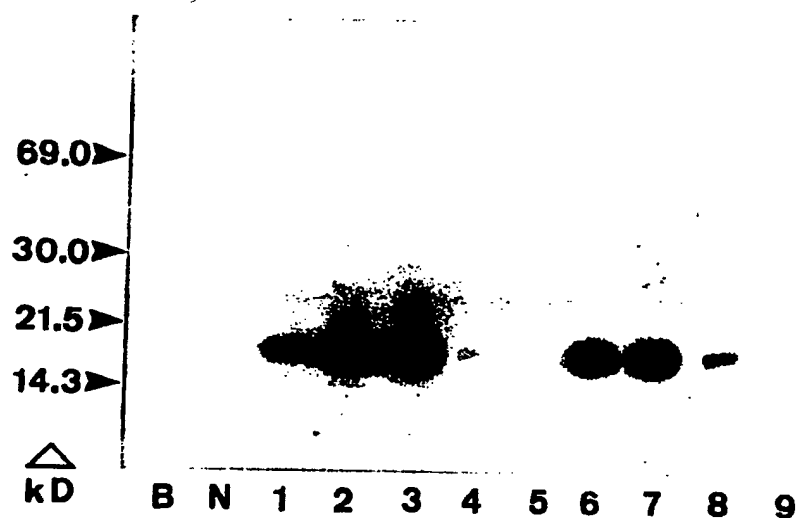


Fig. 3

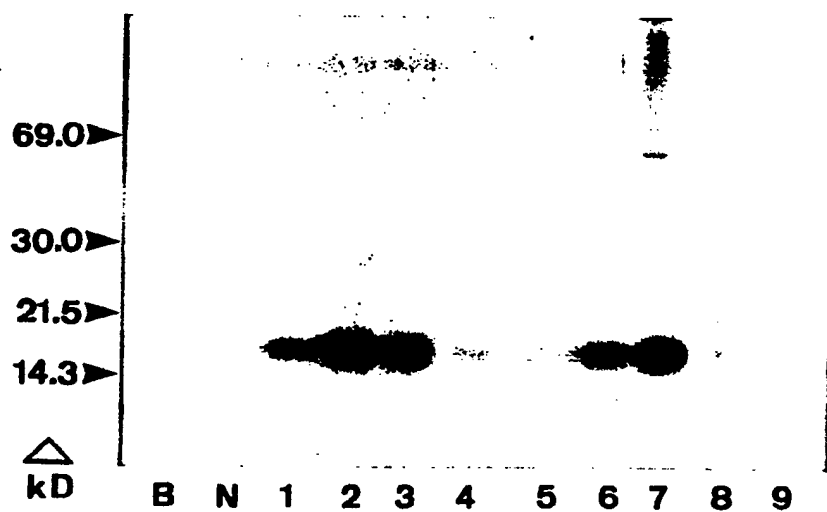
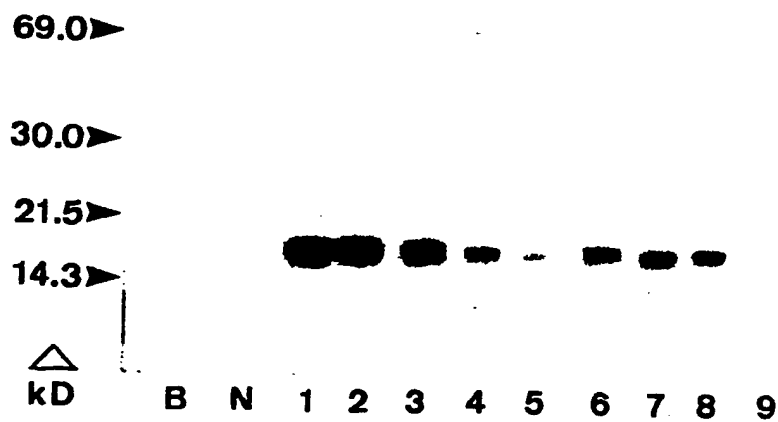
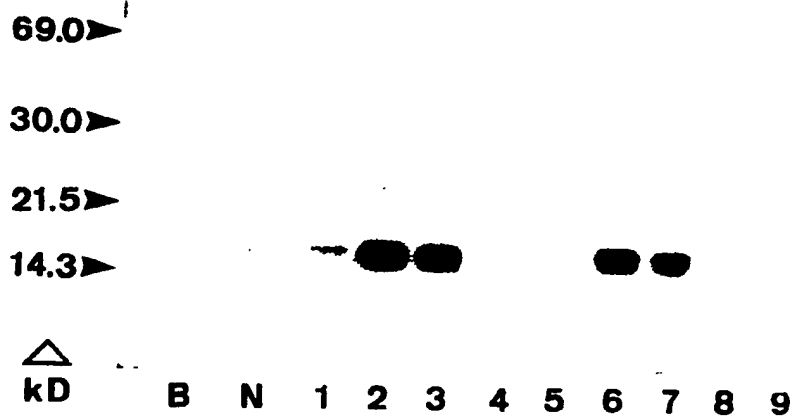
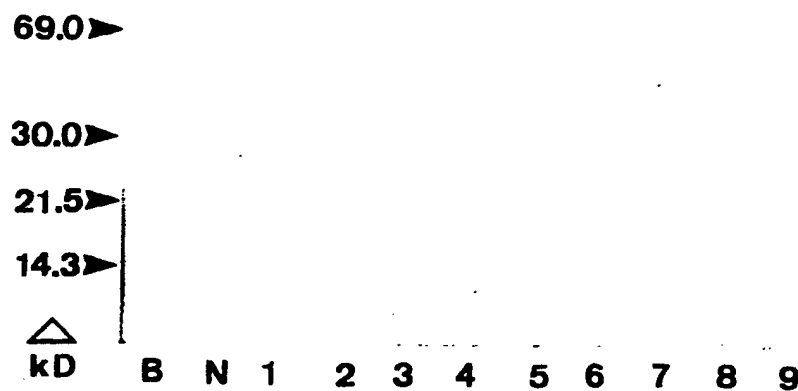
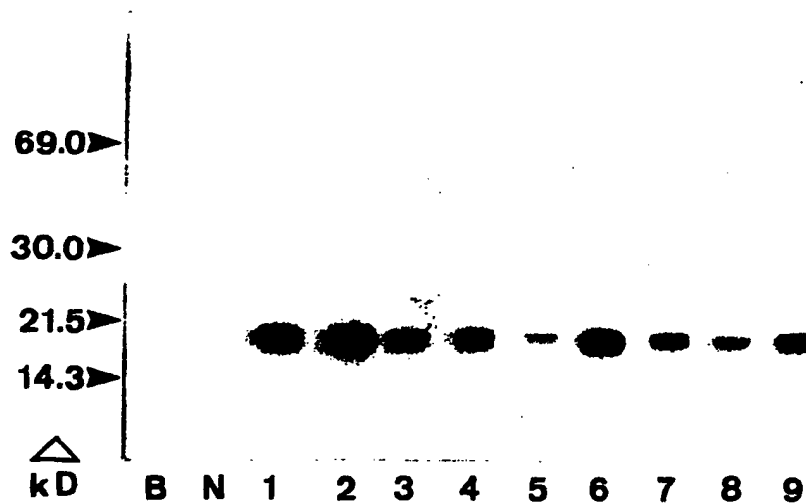
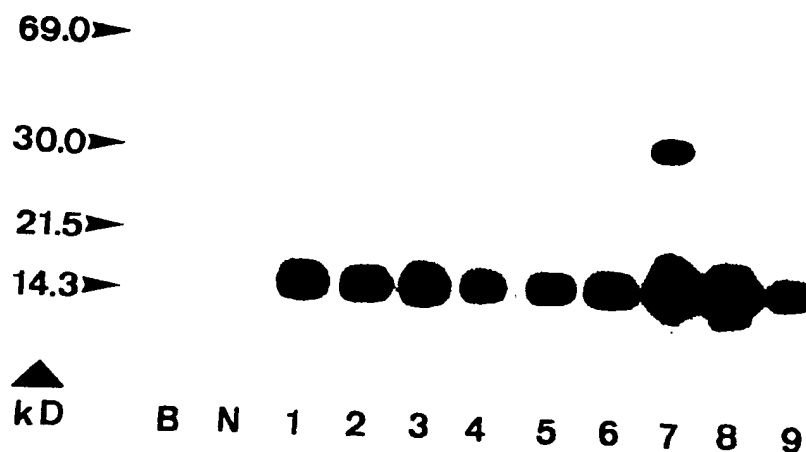


Fig. 4

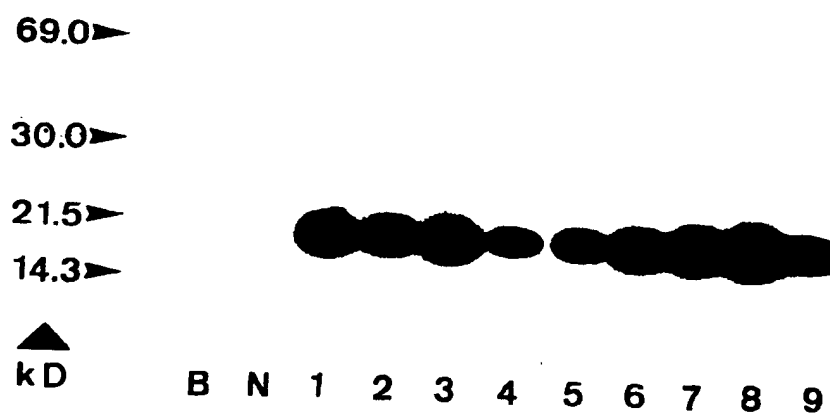
*Fig. 5**Fig. 6*

*Fig. 7**Fig. 8*

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*Fig. 9*



*Fig. 10*

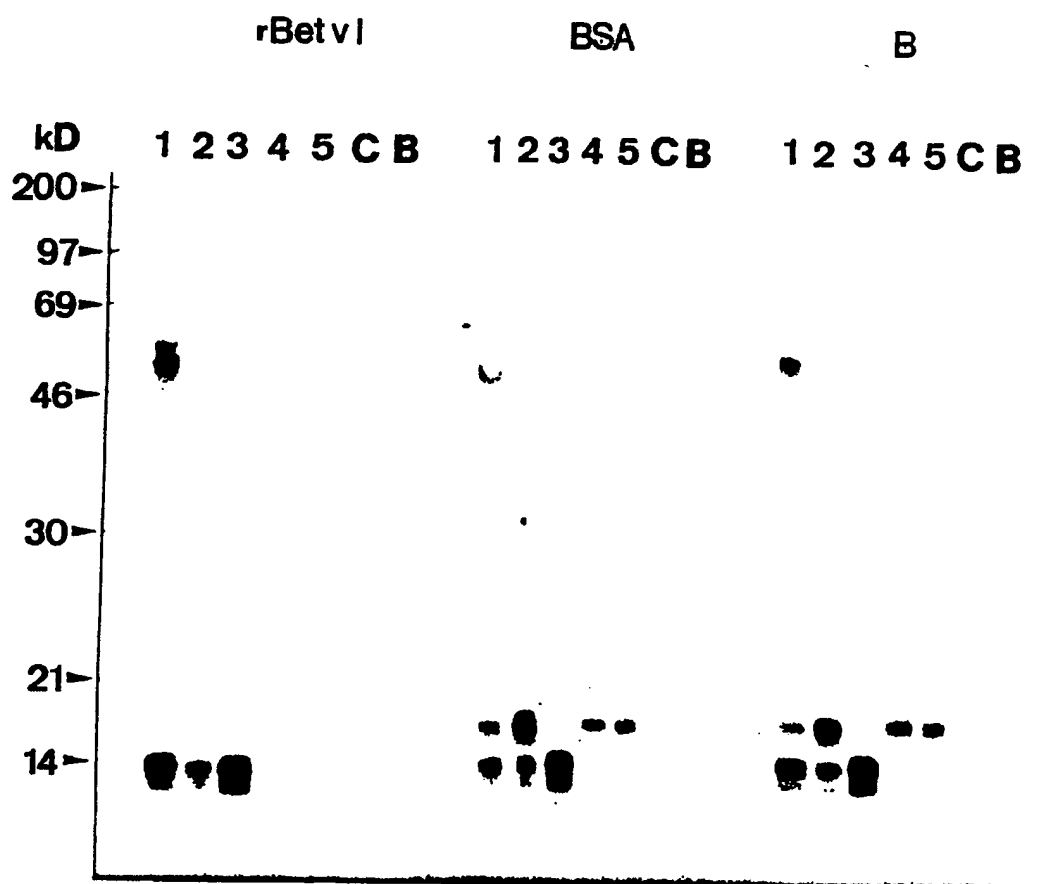


FIG. 11